CENTRAL RESEARCH & DEVELOPMENT DEPARTMENT
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(w/att.)

C-4124

Complainant's Exhibit No. 79

May 12, 1982

MEMO TO: G. L. KENNEDY

FROM : B. C. McKUSICK

ANALYSIS OF BLOOD SERUM FOR FC-143

I attach a 3M procedure for the above analysis. The serum is acidified with reagent grade HCl (presumably 12N) to a concentration of about 2.4N. Within minutes the mixture is extracted with 90% hexane/10% diethyl ether. The hexane/ether extract is evaporated and the residue is treated with ethereal diazomethane. All of these operations are at room temperature except the evaporation, which is at 50°C.

Both the work of Taves several years ago and that of Haskell recently suggest that blood FC-143 is mainly associated with the protein fraction.

It seems to me that the ability to extract FC-143 from serum under the described mild conditions suggests that FC-143 is bound to protein by ionic (acid-base) bonds rather than covalent bonds (amide or perhaps ester bonds). A few minutes of treatment with 2.4N HCl at room temperature seems inadequate to hydrolyze an amide or ester of FC-143.

The acids $CF_3O(CF_2O)_mCF_2CO_2H$ are so similar to FC-143 and other perfluoroalkanoic acids R_fCO_2H in physical and chemical properties that both classes probably bind to protein in similar fashion.

BCM/bjd
Att.

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ANALYSIS OF SERUM FOR FC-143

INTRODUCTION

FC-143 is extracted from an acidified serum sample with hexane/ether and converted with diazomethane (DAM) to the methyl ester for GC.

PREPARATION OF DIAZOMETHANE

Use the Aldrich Chemical Company's procedure for "Preparation of Etheral-Alcoholic Solution of Diazomethane" from DIAZALD as detailed below.

1. Preparation of etheral-alcoholic solutions of diazomethane:

   Ethanol (95%, 25 ml) is added to a solution of potassium hydroxide (5g) in water (8 ml) in a 100 ml distilling flask fitted with dropping funnel and an efficient condenser set downward for distillation. The condenser is connected to two receiving flasks in series, the second of which contains 20-30 ml ether. The inlet tube of the second receiver dips below the surface of the ether, and both receivers are cooled to 0°.

   The flask containing the alkali solution is heated in a water bath to 65°, and a solution of 21.5g (0.1 mole) of Diazald^{R} in about 200 ml of ether is added through the dropping funnel in about 25 minutes. The rate of distillation should about equal the rate of addition. When the dropping funnel is empty, another 40 ml of ether is added slowly and the distillation is continued until the distilling ether is colorless. The combined etheral distillate contains about 3g of diazomethane and must be stored in the freezer compartment of a refrigerator (shelf life is about 2 weeks or until weakly yellow).

   Diazomethane is not only exceedingly toxic, but its solutions have been known to explode quite unaccountably. Hence, ALL WORK WITH DIAZOMETHANE, regardless of how it is generated, SHOULD BE CARRIED OUT BEHIND SAFETY SHIELD IN EFFICIENT HOODS. Use TEFLON sleeves on all ground glass joints.

EXTRACTION

Place up to 20 ml of serum (if less than 20 ml, add water to make a total volume of 20 ml aqueous) in a 50 ml polypropylene screw cap centrifuge tube (Nalgene 3119-0050). Add about 5 ml of reagent grade HCl followed by 15 ml of 90% hexane/10% ether. Shake 2 minutes, high speed centrifuge (12,000 rpm) for 2 minutes and transfer the hexane/ether phase to a glass vial (Kimble 60957).

Repeat extraction twice, each time with 10 ml hexane/ether [it may be necessary to break up centrifuged solids with a stainless steel spatula (Scientific Products, S-1575)]. Evaporate vial's contents at 50°C under a stream of N_2 to 20 µl of C_{10} internal standard (1 µg/µl $C_9F_{19}COOH$ in methanol). Add DAM until under a stream of N_2. Bring up to 5 ml volume with the 90 hexane/10 ether and inject 5 µl into the GC.

GAS CHROMATOGRAPHY

COLUMN

12' (1/8" o.d. ss) 20% DC-200 and 10% Bentone 34 on Anakrom ABS, 100/110 mesh fitted with on-column injection.

PROGRAM

An initial temperature of 50°C is programmed at 4°/min. for 9.2 min., then 10°/min. to a final temperature of 150° with a 6 min. hold. Injection temperature is 200°C, electron capture detector is 340°C with a flow of 28 ml/min. of 95 Ar/5 CH_4.

DISCUSSION

The peak corresponding to the methyl ester of FC-143 will occur at 11.1 min. and the peak for the methyl ester of the C_{10} internal standard will occur at 13.9 min.

Using 3 extractions, one can expect (at the 2-10 µg level) to recover 85 ± 10% of FC-143 as determined (both GC method and with radio-labeled/ scintillation counting standards) by adding known FC-143 to Red Cross plasma samples.

The above method can also be used for human urine analysis. That analysis will be the subject of a separate written procedure.

The internal standard serves the purpose of monitoring the GC injection, the detector response and efficiency of the DAM reaction. It is assumed that 20 µl of the C_{10} standard can be accurately added to the test sample, therefore any variation in resultant GC peak areas for the C_{10} are assumed to be response factors applicable to FC-143. Thus all C_{10} areas are normalized and any necessary factor applied to the FC-143 area permitting one to obtain an accurate FC-143 analysis.

CALIBRATION

A standard solution of FC-143 is prepared by dissolving 100 mg in 100 ml methanol, this is equivalent to 1 µg/µl. As a precaution prior to DAM, 1 drop of p-toluenesulfonic acid (~1% in methanol) is added to assure acid conditions.

J. W. Belisle
/df

cc: D.F. Hagen - 201-1W
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